CHAPTER 5

ACTIVATION OF SEROTONERGIC NEUROTRANSMISSION DURING THE PERFORMANCE OF AGGRESSIVE BEHAVIOUR IN RATS

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ABSTRACT

In general high aggression is thought to be linked to a lowered serotonergic (5-HT) neurotransmission. While this may hold for high aggression as trait characteristic of an individual, there is probably an increased activity of the 5-HT system during the performance of aggressive behaviour. To test this hypothesis two experiments were carried out in male Wildtype Groningen rats. Systemic administration of 5-HT_{1A} or $_{1B}$ receptor agonists has been shown to decrease the overt expression of aggressive behaviour. This is hypothesised to be mediated via autoreceptors. In order to verify this site of action, in the first experiment the effect of local administration of drugs into the dorsal raphe nucleus was studied. After the application of the 5-HT_{1A} agonist alnespirone (25 μg / 0.5 μl) or the $GABA_A$ agonist muscimol (50 ng / 0.5 μl), treatments that inhibit 5-HT neuronal activity, aggressive behaviour was found to be decreased. Second, it was studied to what extent 5-HT neurons in the dorsal and median raphe nuclei had been activated (measured by *c-fos* expression) after a resident-intruder test. In high aggressive rats that had spent almost 50 % of the time on aggressive behaviour, the activation of 5-HT neurons was stronger than in low aggressive rats that showed only very little aggressive behaviour, or in control rats that had no social confrontation. From these results it can be concluded that with performance of aggressive behaviour 5-HT neuronal activity is increased, and that preventing this activation inhibits the expression of aggressive behaviour.

1 INTRODUCTION

A close relation between serotonergic neurotransmission and aggression has long been recognised. In general high levels of aggression are associated with low tonic activity of the 5-HT system (Coccaro, 1989; Coccaro & Astill, 1990; Higley et al., 1996; Kavoussi et al., 1997; Linnoila & Virkkunen, 1992; Mehlman et al., 1994; Popova et al., 1991; Tuinier et al., 1995; Van Praag 1998, Westergaard et al., 1999). Nevertheless it is important to define 'high aggression' carefully: It may stand for the propensity of an individual to react aggressively in many circumstances (aggression as trait characteristic of an individual); on the other hand it may refer to the actual performance of aggressive behaviour (aggression as a state). In the majority of the studies linking high aggression to low 5-HT, aggression is regarded as trait characteristic.

However, the involvement of 5-HT neurotransmission in controlling the performance of aggressive behaviour should be distinguished from the relation between 5-HT and trait aggression. Numerous pharmacological studies have shown that 5-HT_{1A} or $_{1B}$ receptor agonist administration reduces aggressive behaviour (De Boer et al., 1999; Fish et al., 1999; Mos et al., 1993; Olivier et al., 1995). Receptors of both subtypes exist postsynaptically, but also as autoreceptors, the 1A mainly somatodendritic, the 1B at presynaptic terminals. As autoreceptors they are involved in the negative feedback of the 5-HT system (Bonvento et al., 1992; Evrard et al., 1999; Jolas et al., 1995; Kidd et al., 1993; Pineyro & Blier, 1999). De Boer et al. (2000) demonstrated that the anti-aggressive effect of 5-HT_{1A} agonists is mainly mediated via autoreceptors, using S-15535, a 5-HT_{1A} autoreceptor agonist, with antagonistic properties for postsynaptic 5-HT_{1A} receptors. This means that the observed reduction of aggressive behaviour is caused by inhibition of serotonergic activity.

The first aim of the studies presented here was to gain further evidence for involvement of somatodendritic 1A autoreceptors in the anti-aggressive action of 1A agonists. Cell bodies of rostrally projecting 5-HT neurons are located in the dorsal (DR) and median (MR) raphe nuclei. The activity of these neurons can be inhibited via activation of 5-HT_{1A} autoreceptors or $GABA_A$ hetero-receptors (Bonvento et al., 1992; Casanovas et al., 1997; Casanovas et al., 1999; Higgings et al., 1988; Jolas et al., 1995; Kidd et al., 1993; Pineyro & Blier, 1999; Tao et al., 1996, Tao & Auerbach 2000). Therefore the behavioural effect of local administration of the 5-HT_{1A} agonist alnespirone or $GABA_A$ agonist muscimol into the DR was studied.

When inhibition of serotonergic transmission reduces aggressive behaviour,

this suggests that normally activation of the 5-HT system is necessary to express aggression. In a second experiment activation of 5-HT neurons during aggressive behaviour was investigated. After a standard resident-intruder (RI) test *c-fos* expression in 5-HT neurons was examined, in (high aggressive) rats that showed a lot of aggressive behaviour during the test, and in (low aggressive) rats that were hardly aggressive. Another group of high aggressive rats was used without a preceding RI test, to control for possible basal differences between high and low aggressive rats.

2 METHODS

2.1 Animals and housing

For these studies male Wildtype Groningen rats (*Rattus norvegicus*) were used, because these rats exhibit a rich social behaviour, including aggressive behaviour, and there is a high inter-individual variation in genetics, behaviour and physiology. The ancestors were originally caught in the wild, and the strain has been randomly bred in our laboratory for approximately 20 generations.

The rats had free access to food and water throughout the experiments and were housed in climate controlled rooms under a 12:12 h light-dark cycle, all experiments being carried out in the dark phase. After weaning, at the age of 23 days, rats were housed in groups of six males in clear Plexiglas cages (55 x 35 x 20 cm), till they were tested for offensive aggression in a standardised resident-intruder paradigm at 4.5 months of age. Each rat was housed in a cage (80 x 55 x 40 cm) together with a sterilised female to stimulate territorial behaviour and prevent social isolation. Body weight of the rats ranged initially from 370 - 450 g, up to 500 g at the end of the experiments. The experiments have been approved by the animal experiments committee of the University of Groningen.

2.2 Aggression tests

After a week of habituation to the new home cage, four aggression tests were carried out on consecutive days. For each test, the female was removed 30 minutes in advance, and an unfamiliar male conspecific (*Wistar,* body weight ± 350 g) was introduced into the home cage of the experimental rat. For three days the attack latency time (ALT) was scored, the test being terminated shortly after occurrence of an attack, or after a maximum test duration of ten minutes. During the fourth test the full behavioural profile was recorded for ten minutes, using The Observer

(Noldus Information Technology, Wageningen, The Netherlands, version 3.0). The following behavioural elements were scored: aggressive behaviour (clinch, threat, offensive upright, keep down, chase), social behaviour (investigate opponent, sniff in ano-genital region, social groom, mount), explorative behaviour (explore environment, rear), immobility and groom. (See Koolhaas et al., 1980, for a more detailed description of agonistic behaviour).

2.3 Local administration

For this experiment rats were selected with a moderate to high level of aggression, i.e. rats that attacked in each of the four tests and exhibited aggressive behaviour for at least 30% of the time in the fourth test. Guide cannulas (Plastics One Inc., Roanoke, Virginia, USA) were implanted under general anaesthesia of halothane/N_2O/O_2; procaine was administered subcutaneously at the place of incision for local analgesia because of the sensitivity of the membranes on the skull. The guides were placed stereotactically, according to the brain map of Pellegrino & Cushman (1967) (toothbar + 5 mm), for infusion into the DR nucleus (n = 16) at 6.0 mm posterior to bregma and 1.4 mm lateral of the midline, under a 12° lateral angle, and 6.6 mm ventral of the dura mater; for control infusion into the aqueduct (AD; n = 11) guides were placed at 6.6 mm posterior to bregma and 1.4 mm lateral of the midline, under a 12° lateral angle, and 5.5 mm ventral of the dura mater (figure 1). Heightening the toothbar and implanting with a lateral angle were necessary to avoid damaging the sinus.

Rats were allowed to recover for at least two weeks. Each rat was housed in a cage (80 x 55 x 40 cm), the first days single for recovery, then together with a sterilised female. To determine the effect of local administration of drugs on the behaviour, rats were tested twice a week in a standard 10 min resident-intruder test, as described in the previous section. In the first test basal levels of aggression were measured, and in a second test two days later rats received an infusion prior to the aggression test. The rat was taken from the home cage and an infusion cannula was placed in the guide, protruding below its tip for 1 mm. Via the infusion cannula, connected to a Hamilton syringe with a tube (inner diameter 0.28 mm), 0.5 µl fluid was infused in 7 min using an infusion pump. One minute after the end of infusion the infusion cannula was taken out, the dummy was replaced and the rat was placed back in its home cage. Fifteen minutes after the start of infusion an aggression test was carried out. The following infusions were given in random order: vehicle (0.5 µl), the 5-HT_{1A} agonist alnespirone (25 µg / 0.5 µl) or the $GABA_A$ agonist muscimol (50 ng / 0.5 µl). Alnespirone has been used as 5-HT_{1A}

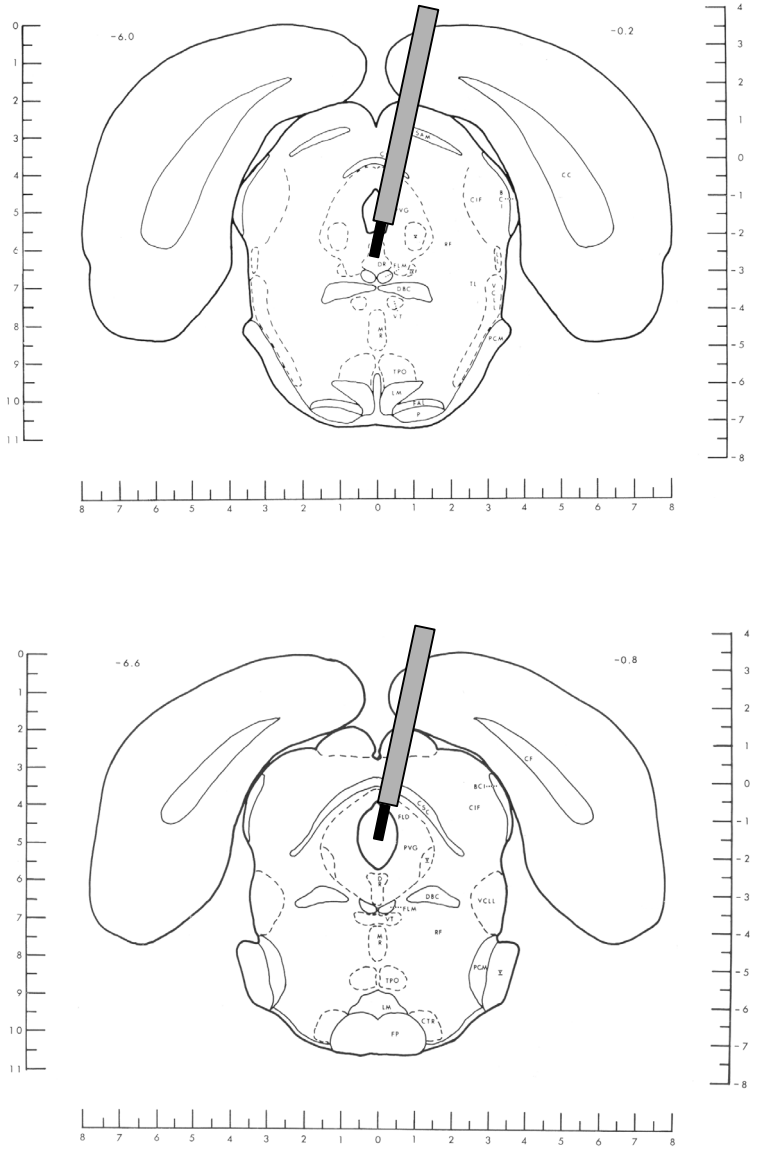

Figure 1: Pictures from the brain map of Pellegrino & Cushman (1967) depicting the location of the cannulas A) in the DR at 6.0 mm posterior to bregma and B) in the AD at 6.6 mm posterior to bregma.

agonist, for its selective anti-aggressive effect (De Boer et al., 1999) Doses were chosen based on published results, about drug administration into the brain, and showing inhibition of 5-HT transmission by local DR administration of muscimol (1 - 100 ng) (Higgins et al., 1988; Nishikawa & Scatton, 1985) or 8-OH-DPAT (5-HT$_{1A}$ agonist, 0.1 - 5 μg) (Bonvento et al., 1992; Jolas et al., 1995). No data were available on DR administration of alnespirone, but 5-HT transmission can be inhibited by systemic administration of 8-OH-DPAT (0.025 - 0.3 mg/kg) or alnespirone (0.1 - 3 mg/kg) (Casanovas et al., 1997) indicating that from alnespirone a higher dose is required. In a pilot experiment 5 μg alnespirone was administered into the DR, causing a small, non significant decrease in aggressive behaviour (38.86 ± 2.65 resp. 33.72 ± 2.22 % of the time) and an almost significant increase in ALT (64 ± 9 resp. 225 ± 57 sec, *t*-test: p=0.067, n=5). Therefore a five-fold higher dose of 25 μg was applied in this experiment, that has been carried out in different cohorts.

Alnespirone [(+)-S-20499-2 hydrochloride; lot no. 48647, molecular weight 479] was provided by Institut de Recherches Internationales Servier, France. Muscimol [5-aminomethyl-3-hydroxyisoxazole; lot no. 6/11143, molecular weight 114.10] was obtained from Tocris, Bristol, UK. Both drugs were dissolved in ultra pure water (vehicle solution).

2.4 c-fos expression

In order to compare the effects of a differential amount of aggressive behaviour, rats were tested for aggression as described in section 2.2, and high aggressive and low aggressive individuals were selected (see results section; n = 7 and 6 resp.). They were perfused two hours after the start of a standard RI test. A control group of high aggressive rats (n = 7) was perfused without a preceding aggression test. All subjects were housed in the same room and taken in random order. They were perfused under deep anaesthesia (1 ml pentobarbital, intraperitoneally) with heparinised saline (10 ml heparin / l saline) for 1 min, followed by 4% paraformaldehyde in 0.1 M phosphate buffer containing 0.1% $MgSO_4$, pH 7.4. Brains were removed, post-fixed overnight in 4% paraformaldehyde solution at 4 °C, and stored in 0.1 M phosphate buffered saline (PBS) containing 0.1% azide, at 4 °C. After dehydration in 30% sucrose solution (0.1 M phosphate buffer + 0.1% azide) for 48 - 72 hours brains were sliced in coronal sections of 30 μm using a cryostat microtome. Free-floating sections were immunostained for 5-HT and c-fos.

In order to identify the 5-HT neurons, sections were incubated, after preincubation in 5% normal goat serum (NGS), with the primary antibody (1:200

rabbit anti 5-HT, Zymed, San Francisco, California, USA) for two hours at room temperature and two days at 4°C, followed by incubation with biotinylated goat anti rabbit immunoglobulin G (1:200, Zymed) for two hours at room temperature; all in 0.01 M PBS (containing 0.05% Tween and 1% NGS, and 0.1% azide for periods longer then a day) with intermittent rinsing in PBS/Tween. Then sections were incubated with streptavidin-alkaline phosphatase (1:100, Zymed, in 0.05 M Tris buffered saline) for one hour at room temperature. Subsequently the sections were coloured for a maximum of 30 min in Fuchsin solution (300 µl fuchsin solution of 5 g fuchsin in 100 ml 2 N HCl, 150 µl 4% sodium nitrite, 60 ml 0.05 M Tris buffer, 60 µl 1 M levamisol, 2 ml naphtol-AS-BI-phosphate solution [18 mg in 2 ml dimethylformamide], pH 8.0 - 8.4).

Then sections were stained for *c-fos*: After pretreatment with 0.3% H_2O_2 and preincubation in 5% NGS sections were first incubated with primary antibody rabbit anti fos (1:8000, Santa Cruz Biotechnology, California, USA) for two days, followed by a biotinylated goat anti rabbit immunoglobulin G (1:200, Zymed) for two hours, and streptavidin-horseradish peroxidase complex (1:200, Zymed) for one hour, all in 0.01 M PBS, with intermittent rinsing also in 0.01 M PBS. After rinsing in 0.05 M Tris-HCl sections were incubated with nickel-diaminobenzidine for five minutes, and the colouring reaction was started adding 1% H_2O_2 and stopped by rinsing with Tris-HCl after 15 minutes.

Sections were mounted on slides. Fos positive cells, 5-HT positive neurons and double-labelled neurons in the DR and MR were counted manually within a 1000 x 1000 µm grid at a magnification of 200 in two sections per rat, by someone blind to the identity of the animal The location and relative size of the analysed areas are indicated in figure 2.

2.5 Statistical analysis

Data of the local administration experiment were tested with an ANOVA for repeated measurements for the behavioural categories and ALT separately, with test (basal vs. infusion) and treatment (vehicle - alnespirone - muscimol) as within subject factors, and group (DR or AD) as between subject factor. Posthoc pairwise comparisons (LSD test) were done based on estimated marginal means.

Behavioural data of the *c-fos* expression experiment were analysed with ANOVA: For confirmation of the intended differences and similarities in aggressiveness between the experimental groups the behavioural data of the selection tests were statistically analysed after division. For ALT data an ANOVA for repeated measurements was used, with test (1-4) as within subject factor and

group (high aggressive - low aggressive - control) as between subject factor, followed by posthoc pairwise comparisons (LSD). The behavioural categories of the fourth selection test were analysed separately with group (high aggressive - low aggressive - control) as between subject factor, followed by a post hoc LSD test. For analysis of the test before perfusion an ANOVA for repeated measurements was used for the ALT (the high and low aggressive group as between subject factor and 5 tests as within subject factor) followed by pairwise comparisons; and a two-way ANOVA for the behavioural categories (two tests as within subject factor and the high and low aggressive group as between subject factor). The results of staining were analysed with a *t*-test for independent samples.

For all tests the software package SPSS was used, version 10.0.5 and in all cases differences were regarded to be significant when $p < 0.05$. Data are expressed as mean ± sem.

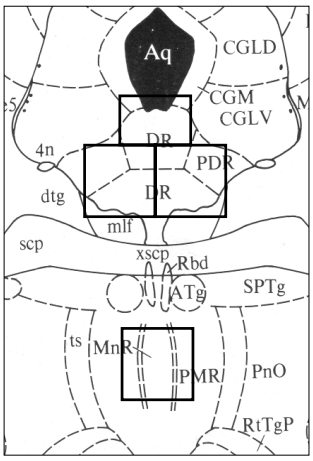

Figure 2: Location and relative size of the areas in which 5-HT and c-fos positive cells were counted in the DR and MR, approximately 8 mm posterior to bregma (Paxinos & Watson, 1986).

3 RESULTS

3.1 Local administration

Histological verification of the location of infusion sites showed that in two cases the tip of the DR cannula was not in a proper position; data of these rats were excluded from the results. In all other cases cannulas were correctly placed.

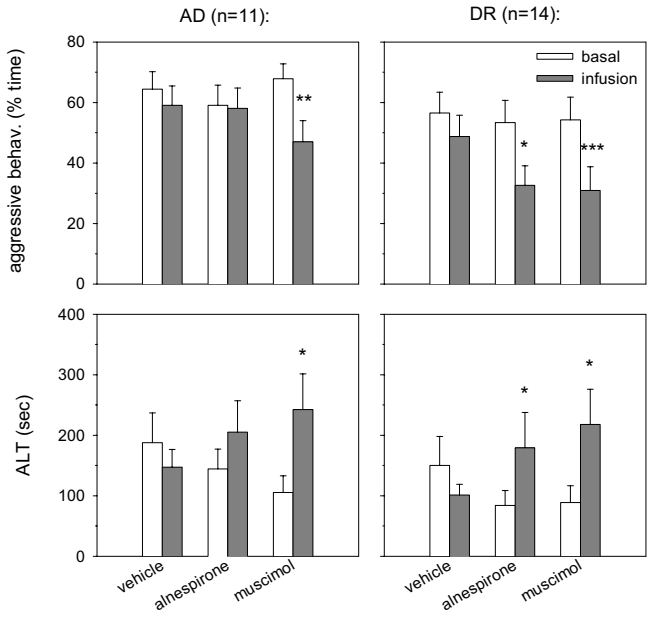

*Figure 3: Effect of administration of drugs into the DR or AD (as control) on aggressive behaviour, measured as: percentage of the time spent on aggressive behaviour and attack latency time (ALT). LSD test: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.*

Administration of alnespirone or muscimol into the DR lowered aggressive behaviour significantly, measured as a decrease in time spent on aggressive behaviours and an increased ALT, while vehicle administration did not affect aggressive behaviour (figure 3). Infusing vehicle or alnespirone into the AD had no effect on aggression, but infusing muscimol lowered aggression in the AD group as well. Analysing the percentage time spent on aggressive behaviour with ANOVA resulted in a significant test effect [$F(1,23)=27.315$; $p<.001$]. Pairwise comparisons

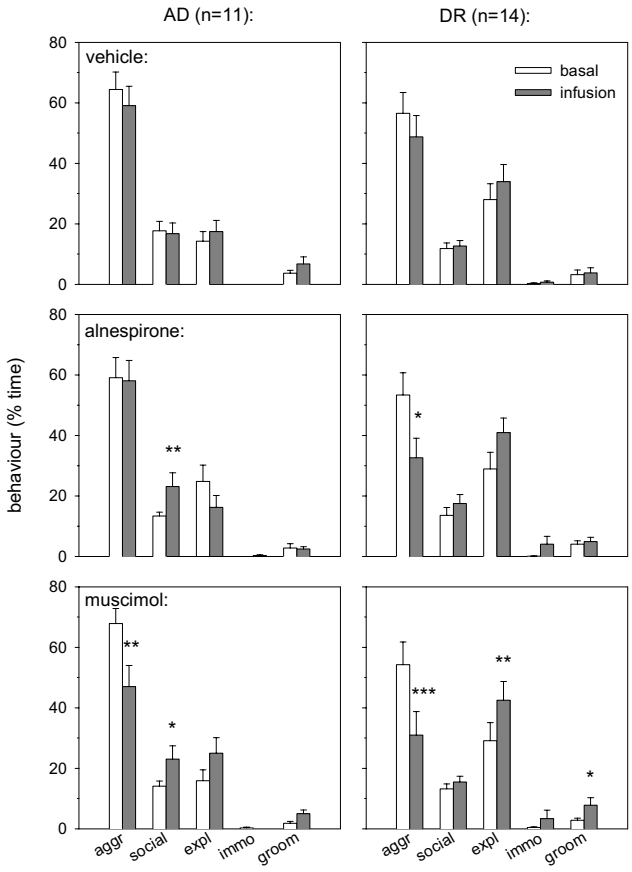

*Figure 4: Effect of administration of drugs into the DR or AD (as control) on the total behavioural profile. The behavioural elements were classified in the following categories: aggressive behaviour, social behaviour, explorative behaviour, immobility, grooming. LSD test: * = p < 0.05, ** = p < 0.01, *** = p < 0.001.*

showed that there were no differences in basal values between groups or between the different treatments. There were differences between the test after infusion compared to the preceding basal test in the DR group for alnespirone [p=.015] and muscimol [p<.001], and in the AD group for muscimol [p=.002]. Analysis of the ALT resulted in an effect of test [F(1,23)=8.142; p=.009] and a test x treatment interaction [F(2,46)=7.524; p=.001]. Pairwise comparisons revealed no differences

in basal values between groups or between different treatments, while the infusion test differed significantly from the basal test after alnespirone [p=.025] and muscimol [p=.024] administration into the DR, and after muscimol [p=.032] administration into the AD.

In figure 4 the effect of drug administration on all behavioural categories is shown. Data were analysed per category. For social behaviour there was a main effect of test [F(1,23)=10.163; p=.004] and a test x treatment interaction [F(2,46)=3.943; p=.026]. There were no differences in basal values between groups or between different treatments, while the infusion test differed significantly from the basal test after alnespirone [p=.006] and muscimol [p=.012] administration in the AD group. For explorative behaviour main effects of test [F(1,23)=11.279; p=.003] and group [F(1,23)=7.106; p=.014] and an interaction of test x group [F(1,23)=7.041; p=.014] were found. The basal values differed only for the vehicle test between the groups [p=.045], values after the infusions also differed between groups [vehicle: p=.032; alnespirone: p=.001; muscimol: p=.046] and the infusion test was significantly different from the basal test after muscimol infusion into the DR [p=.004]. For immobility no main effects were found. For grooming there was a test effect [F(1,23)=4.622; p=.042]. There were no differences between basal values, but muscimol administration in the DR caused a difference in percentage time grooming compared to the basal test [p=.030].

3.2 c-fos expression

Rats were selected for this experiment on the basis of the behaviour during four consecutive RI tests. High and low aggressive rats were selected, and in figure 5 the ALT's of the different groups are shown, as well as the full behavioural profile during the fourth test. Low aggressive rats did not attack and showed only very little aggressive behaviour (4.79 ± 2.53 % of the time). The high aggressive and control rats on the other hand did attack, and attacked quicker in the course of time, and they were much more aggressive (resp. 43.60 ± 8.61 and 32.17 ± 3.97 % of the time). Analysis of the results of ALT with ANOVA for repeated measurements resulted in a significant effect of test [F(3,51)=14.173; p<.001] and group [F(1,17)=146.756; p<.001] and an interaction of test x group [F(6,51)=4.342; p=.001]. Posthoc pairwise comparisons showed that the low aggressive rats differed significantly from the high aggressive and control rats in all four tests [p<.001], and that only in test two the high aggressive rats differed from the control rats [p=.046]. During the fourth test there was a difference between groups only for aggressive behaviour (ANOVA:significant effect of group [F(2,17)=11.378;p=.001]): The amount

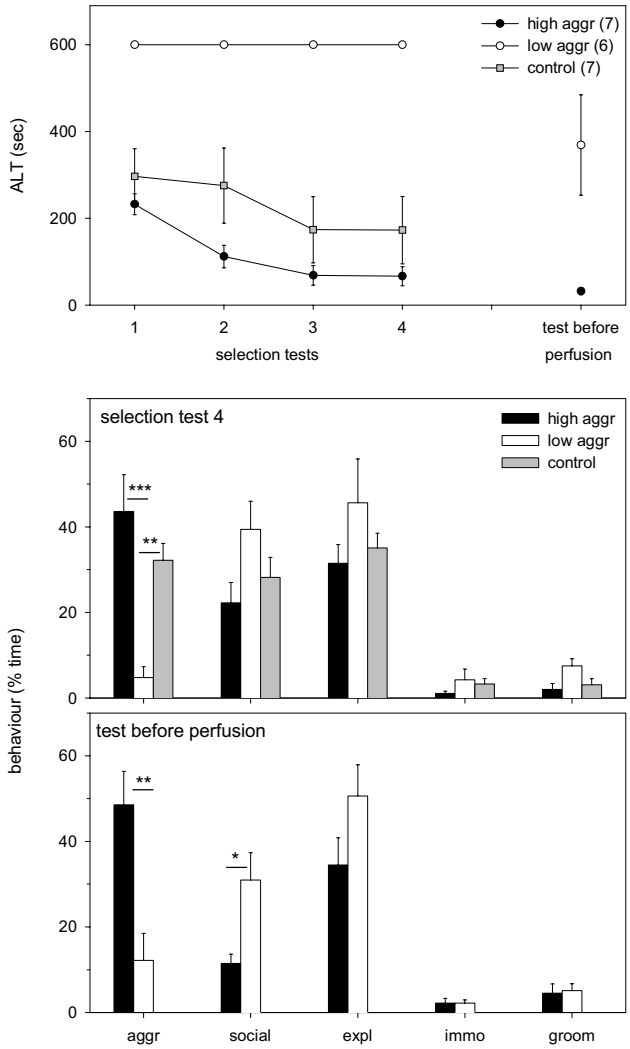

*Figure 5: Behaviour of the high aggressive, low aggressive and control rats in the c-fos expression experiment. On the basis of the ALT in four consecutive RI tests and the full behavioural profile during the fourth test, rats were selected for the different groups. The behaviours and ALT during the test before perfusion are also shown for the high and low aggressive rats. The observed behavioural elements were classified in the following categories: aggressive behaviour, social behaviour, explorative behaviour, immobility, grooming. LSD test: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.*

of aggressive behaviour of the low aggressive rats was less than that of the high aggressive and control rats (post hoc LSD test: p<.001 and p=.004 respectively), while the last two groups did not differ significantly.

Before perfusion only the high and low aggressive rat were tested again in a RI test. Although some of the low aggressive rats did attack, there was still a very clear difference in the level of aggression between the groups: The ALT was longer in the low aggressive group (p=.009 in the pairwise comparison after ANOVA with an effect of test [F(4,44)=10.035; p<.001], group [F(1,11)=250.317; p<.001] and a test x group interaction [F(4,44)=3.488; p=.015]). The low aggressive group also spent a smaller amount of time on aggressive behaviour (p=.005 in the pairwise comparison, after ANOVA revealed a significant effect of group [F(1,11)=17.373; p=.002]), but a larger amount on social behaviour (p=.011, after a group effect in the ANOVA [F(1,11)=5.101; p=.045]). There were no differences in behaviour found within groups between the test before perfusion and the fourth selection test. Only the ALT in the low aggressive group was decreased (p=.014).

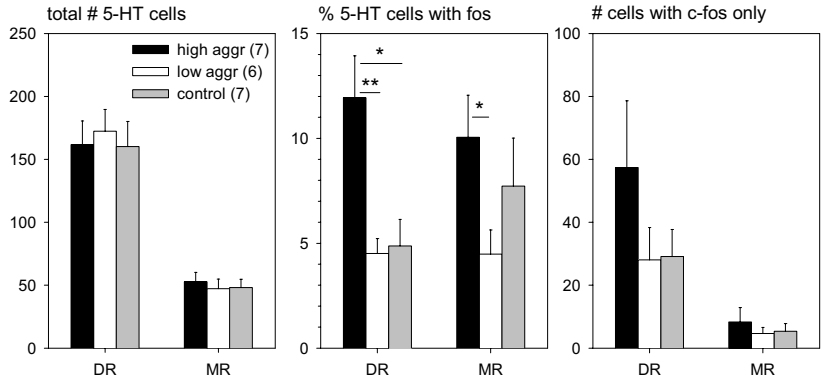

*Figure 6: Total number of 5-HT neurons, percentage of these 5-HT neurons expressing c-fos, and number of non-5-HT neurons expressing c-fos in the DR and MR of high aggressive, low aggressive and control rats. Independent sample T-test: * = p < 0.05, ** = p < 0.01.*

The total number of 5-HT neurons within the analysed areas (figure 2), the percentage of these 5-HT neurons expressing *c-fos*, and the number of non 5-HT

cells expressing *c-fos* are shown in figure 6. Within the DR and the MR there were no differences between groups in the number of 5-HT neurons. A higher percentage of these neurons was activated in the high aggressive rats after a RI test, in the DR compared to low aggressive or control rats (*t*-test: p=.007 resp. p=.011), and in the MR compared to the low aggressive rats (*t*-test: p=.043). There were no significant differences in the number of non 5-HT neurons expressing *c-fos*.

4 Discussion

The main results of this paper are, first, that aggressive behaviour is inhibited by administration of a 5-HT_{1A} agonist or $GABA_A$ agonist in the DR; and second, that there is an increased activation of 5-HT neurons during aggressive behaviour.

The aim of the first experiment was to pharmacologically inhibit 5-HT neuronal activity, and to examine the influence on (aggressive) behaviour. For this purpose the 5-HT_{1A} agonist alnespirone was given within the DR to activate specifically 5-HT_{1A} somatodendritic autoreceptors. This results in an inhibition of 5-HT neurons (Bonvento et al., 1992; Casanovas et al., 1997; Casanovas et al., 1999; Higgins et al., 1988; Jolas et al., 1995; Kidd et al., 1993; Pineyro & Blier, 1999; Tao & Auerbach, 2000). The activity of 5-HT neurons is controlled via $GABA_A$ hetero-receptors as well (Higgins et al., 1988; Pineyro & Blier, 1999; Nishikawa & Scatton, 1985; Tao et al., 1996; Tao & Auerbach, 2000). The $GABA_A$ agonist muscimol was also administered to reach the same physiological effect via different pharmacological routes. Both treatments resulted in a decline of aggressive behaviour, measured as an increased ALT and a decreased percentage of the time the rats show aggressive behaviours.

The effects on behaviour of locally administered drugs are specific: The reduction in aggressive behaviour was compensated by an increase in other behavioural elements, like social or explorative behaviours, without an increase in immobility. And since the vehicle administration had no influence, we may conclude that the procedure itself did not affect behaviour. Another factor to keep in mind when interpreting results of local administration experiments is that the effects of drugs may be mediated in other brain areas than where they are administered, for instance by diffusion of the drug through the brain tissue (Bonvento et al., 1992; Jolas et al., 1995). In the control group of this experiment drugs were administered into the AD, close to the location of the DR. Via the AD the drugs could easily disperse, and this was the most likely route for accidental

leakage from the DR. When spreading of the drug would have taken place in the experimental group, and the observed effects could consequently have been mediated via another brain region, then we expected to see the same effects in the control group. However, alnespirone inhibited aggressive behaviour when applied within the DR, but not in the AD. From this we may conclude that the mechanism via which alnespirone exerts its anti-aggressive effect is via the 5-HT$_{1A}$ autoreceptor. Muscimol had an anti-aggressive effect as well, not only when given within the DR, but also in the AD. Compared to the results of alnespirone administration it is likely that in the DR group the infused drugs act within this nucleus. Moreover, GABA$_A$ receptors are found on the cell bodies and dendrites of 5-HT neurons in the raphe nuclei (Gao et al., 1993), but not or only very little on 5-HT terminals (Mennini et al., 1986). Tao et al. (1996; 2000) showed that administration of the GABA$_A$ agonist muscimol within the DR caused a diminished release of 5-HT, while administration in a 5-HT target area had no effect on the release. The authors conclude that GABA has a tonic inhibitory control function on the 5-HT system via somatodendritic GABA$_A$ hetero-receptors. So, although alternatives cannot fully be excluded, it is likely that the behavioural effects of muscimol administered within the DR are (at least partly) mediated via activation of GABA$_A$ receptors on 5-HT neurons. One possible explanation for the action of muscimol when administered into the AD, is that via other non 5-HT neuronal pathways the same behavioural effect is reached, since the GABA-ergic system is a very widespread inhibitory system in the central nervous system. Depaulis and Vergnes (1983) found an increased aggression after intracerebroventricular administration of a GABA$_A$ agonist and decreased aggression after administration of an antagonist. This indicates a complex relationship between aggressive behaviour and the GABAergic system, partly via the 5-HT system, partly apart from it. Altogether we can conclude that inhibition of 5-HT neuronal activity, via differential pharmacological routes, causes a decrease in aggressive behaviour.

The second experiment aimed to test whether there is an activation of 5-HT neurons during the performance of aggressive behaviour. The results demonstrate that after a confrontation with an intruder rat in a standardised RI test, there is a stronger activation in the rats that showed a large amount of aggressive behaviour, compared to those that showed only little aggressive behaviour. An increased *c-fos* expression could be the result of behavioural activation in general. The low aggressive rats were also behaviourally activated during the test, as can be seen in high scores for social and explorative behaviours, but were less aggressive. It is impossible to match the groups for an exactly equal level of activity, or use a

control group solely for physical activity. The influence of physical effort involved in the performance of aggressive behaviour on enhanced *c-fos* expression cannot be excluded, and may even be very likely to have contributed (Jacobs & Fornal, 1999). However, we may conclude that it is the performance of aggressive behaviour that is associated with increased *c-fos* expression in 5-HT neurons.

In order to obtain a differential level of aggression in the test before perfusion, we had to select high and low aggressive rats beforehand. To exclude a bias of a basal difference between these individuals a control group of high aggressive rats was used, that was not confronted with an intruder. The difference between high aggressive animals with and without a preceding RI test demonstrates that it is the performance of aggressive behaviour that causes the increased *c-fos* expression. Differences between groups are most prominent in the DR, compared to the MR. This may be caused by the higher number of 5-HT neurons counted in the DR, which makes differences in percentages clearer. And in general the involvement of 5-HT neurons within the DR in regulation of aggressive behaviour is widely accepted, while the precise role of MR 5-HT neurons is less clear.

The finding of decreased aggression after local drug application is in line with other studies describing administration of 5-HT$_{1A}$ or $_{1B}$ agonists either systemically (De Almeida et al., 2001; De Boer et al., 1999; De Boer et al., 2000; Fish et al., 1999; Olivier et al., 1995; Sijbesma et al., 1991), or locally into the DR (Mos et al., 1993) or intracerebroventricularly (De Almeida & Lucion, 1994; Mos et al., 1992). In most cases this is explained to be mediated via postsynaptic receptors, e.g. based on studies with neurotoxic destruction of 5-HT neurons (De Almeida et al., 2001; Sijbesma et al., 1991). However this manipulation is non-specific and data are difficult to interpret. In general it is hard to distinguish autoreceptor and postsynaptic receptor mediated processes, in an experimental approach. A complicating side effect of administration of many 5-HT$_1$ agonists is their sedative effect and induction of the so-called "5-HT behavioural syndrome" (De Boer et al., 1999; Higgins et al., 1988; Higgins & Elliott, 1991; Olivier et al., 1995). When Mos et al. (1993) conclude that the anti-aggressive effect of intra-raphe administration of 5-HT$_1$ agonists is mediated via the autoreceptors, it is also a non-specific reduction in aggression. We used the 5-HT$_{1A}$ agonist alnespirone for its selective anti-aggressive effect, i.e. without inducing immobility (De Boer et al., 1999). In this experiment the reduction in aggressive behaviour was not accompanied by an enhanced immobility, but was compensated by increased social and non-social exploration. From these data and previous findings (De Boer et al.,

2000) we may conclude that the selective anti-aggressive effect is mediated via 5-HT_{1A} autoreceptors, and thereby inhibition of 5-HT neurons.

This is in agreement with the observed activation of 5-HT neurons during the performance of aggressive behaviour. Other studies also describe an increased *c-fos* mRNA expression in the DR after an aggressive encounter (Kollack-Walker et al., 1997; Stork et al., 1997). And Delville et al. (2000) reported an enhanced activation of 5-HT neurons after a fight, although they explain this finding in terms of physical activity, because 5-HT is thought to inhibit aggression. This neuronal activation does not seem to be specific for offensive aggressive behaviour, the same circuitry (or parts of it) may also be activated with the performance of other behaviours. Nevertheless, all results suggest an activation of 5-HT neurons. It has been attempted to measure directly changes in neurotransmission related to behaviour. The results of Van Erp & Miczek (2000) measured via microdialysis, of a decreased 5-HT level in the prefrontal cortex following an aggression test, seem to be a contradiction with our results. Unfortunately also this technique is restricted in its limited time resolution, as already mentioned by the authors. Changes in neurotransmission related to behaviour probably occur in very short time span, of seconds rather than minutes (Jacobs & Fornal, 1999). It may therefore not be justified to match neurotransmitter levels measured via microdialysis directly to anticipation, initiation, execution or termination of specific behaviours. The *c-fos* expression studies alone do not allow a differentiation between the performance of behaviour or the recovery, since brains are studied 1 - 2 hours after the behaviour has taken place. A combination of these results leads to the hypothesis of a short lasting activation of 5-HT neurotransmission during the initiation and/or performance of aggressive behaviour, followed by an inhibition.

Previous experiments in our laboratory with high and low aggressive mice and rats demonstrated an increased sensitivity of postsynaptic 5-HT_{1A} receptors in individuals with a high trait aggression, compared to those with a low trait aggression. This could be due to a tonically lower 5-HT transmission in these high aggressive individuals, in line with the general view on 5-HT and aggression as mentioned in the introduction (Van der Vegt et al., 2001). On the other hand the present results make clear this cannot automatically be extrapolated to the involvement of 5-HT transmission in aggressive behaviour.

Summarising, the results of both experiments described in this paper demonstrate that with performance of aggressive behaviour 5-HT neuronal activity is increased, and that preventing this activation is inhibiting the performance of

aggressive behaviour. This leads to the conclusion that, although high trait aggression may be related to a diminished functioning of the central 5-HT system, the overt expression of aggressive behaviour is accompanied by increase in activity of this system.

REFERENCES

1. Bonvento G, Scatton B, Claustre Y, Rouquier L (1992) Effect of local injection of 8-OH-DPAT into the dorsal or median raphe nuclei on extracellular levels of serotonin in serotonergic projection areas in the rat brain. Neurosci.Lett., 137, 101-104

2. Casanovas JM, Lesourd M, Artigas F (1997) The effect of the selective 5-HT1A agonists alnespirone (S-20499) and 8- OH-DPAT on extracellular 5-hydroxytryptamine in different regions of rat brain. Br.J.Pharmacol., 122, 733-741

3. Casanovas JM, Vilaro MT, Mengod G, Artigas F (1999) Differential regulation of somatodendritic serotonin 5-HT1A receptors by 2-week treatments with the selective agonists alnespirone (S-20499) and 8-hydroxy-2-(Di-n-propylamino)tetralin: microdialysis and autoradiographic studies in rat brain. J.Neurochem., 72, 262-272

4. Coccaro EF (1989) Central serotonin and impulsive aggression. Br.J.Psychiatry Suppl, 52-62

5. Coccaro EF, Astill JL (1990) Central serotonergic function in parasuicide. Prog. Neuropsychopharmacol. Biol.Psychiatry, 14, 663-674

6. De Almeida RMM, Lucion AB (1994) Effects of intracerebroventricular administration of 5-HT receptor agonists on the maternal aggression of rats. Eur.J.Pharmacol., 264, 445-448

7. De Almeida RMM, Nikulina EM, Faccidomo S, Fish EW, Miczek KA (2001) Zolmitriptan - a 5-$HT_{1B/D}$ agonist, alcohol, and aggression in mice. Psychopharmacology, 157, 131-141

8. De Boer SF, Lesourd M, Mocaer E, Koolhaas JM (1999) Selective antiaggressive effects of alnespirone in resident-intruder test are mediated via 5-hydroxytryptamine1A receptors: A comparative pharmacological study with 8-hydroxy-2-dipropylaminotetralin, ipsapirone, buspirone, eltoprazine, and WAY-100635. J.Pharmacol.Exp.Ther., 288, 1125-1133

9. De Boer SF, Lesourd M, Mocaer E, Koolhaas JM (2000) Somatodendritic 5-HT(1A) autoreceptors mediate the anti-aggressive actions of 5-HT(1A) receptor agonists in rats: an ethopharmacological study with S-15535, alnespirone, and WAY-100635. Neuropsychopharmacology, 23, 20-33

10. Delville Y, De Vries GJ, Ferris CF (2000) Neural connections of the anterior hypothalamus and agonistic behavior in golden hamsters. Brain Behav.Evol., 55, 53-76

11. Depaulis A, Vergnes M (1983) Induction of mouse-killing in the rat by intraventricular injection of a GABA-agonist. Physiology & Behavior, 30, 383-388

12. Evrard A, Laporte AM, Chastanet M, Hen R, Hamon M, Adrien J (1999) 5-HT1A and 5-HT1B receptors control the firing of serotoninergic neurons in the dorsal raphe nucleus

of the mouse: studies in 5-HT1B knock-out mice. Eur.J.Neurosci., 11, 3823-3831

13. Fish E.W, Faccidomo S, Miczek KA (1999) Aggression heightened by alcohol or social instigation in mice: reduction by the 5-HT(1B) receptor agonist CP-94,253. Psychopharmacology (Berl), 146, 391-399

14. Gao B, Fritschy JM, Benke D, Mohler H (1993) Neuron-specific expression of GABAA-receptor subtypes: differential association of the alpha 1- and alpha 3-subunits with serotonergic and GABAergic neurons. Neuroscience, 54, 881-892

15. Higgins GA, Bradbury AJ, Jones BJ, Oakley NR (1988) Behavioural and biochemical consequences following activation of 5HT1- like and GABA receptors in the dorsal raphe nucleus of the rat. Neuropharmacology, 27, 993-1001

16. Higgins GA, Elliott PJ (1991) Differential behavioural activation following intra-raphe infusion of 5- HT1A receptor agonists. Eur.J.Pharmacol., 193, 351-356

17. Higley JD, King ST, Hasert MF, Champoux M, Suomi SJ, Linnoila M (1996) Stability of interindividual differences in serotonin function and its relationship to severe aggression and competent social behavior in rhesus macaque females. Neuropsychopharmacology, 14, 67-77

18. Jacobs BL, Fornal CA (1999) Activity of serotonergic neurons in behaving animals. Neuropsychopharmacology, 21, 9S-15S

19. Jolas T, Schreiber R, Laporte AM, Chastanet M, De Vry J, Glaser T, AdrienJ, Hamon M (1995) Are postsynaptic 5-HT1a receptors involved in the anxiolytic effects of 5-HT1a receptor agonists and in their inhibitory effects on the firing of serotonergic neurons in the rat? The journal of pharmacology and experimental therapeutics, 272, 920-929

20. Kavoussi R, Armstead P, Coccaro E (1997) The neurobiology of impulsive aggression. Psychiatr.Clin.North Am., 20, 395-403

21. Kidd EJ, Haj-Dahmane S, Jolas T, Lanfumey L, Fattaccini CM, Guardiola-Lemaitre B, Gozlan H, Hamon M (1993) New methoxy-chroman derivatives, 4[N-(5-methoxy-chroman-3-yl)N- propylamino]butyl-8-azaspiro-(4,5)-decane-7,9-dione [(+/-)-S 20244] and its enantiomers, (+)-S 20499 and (-)-S 20500, with potent agonist properties at central 5-hydroxytryptamine1A receptors. J.Pharmacol.Exp.Ther., 264, 863-872

22. Kollack-Walker S, Watson SJ, Akil H (1997) Social stress in hamsters: defeat activates specific neurocircuits within the brain. J.Neurosci., 17, 8842-8855

23. Koolhaas JM, Schuurman T, Wiepkema PR (1980) The organization of intraspecific agonistic behaviour in the rat. Prog.Neurobiol., 15, 247-268

24. Linnoila VM, Virkkunen M (1992) Aggression, suicidality, and serotonin. J.Clin.Psychiatry, 53 Suppl, 46-51

25. Mehlman PT, Higley JD, Faucher I, Lilly AA, Taub DM, Vickers J, Suomi SJ, Linnoila M (1994) Low CSF 5-HIAA concentrations and severe aggression and impaired impulse control in nonhuman primates. Am.J.Psychiatry, 151, 1485-1491.

26. Mennini T, Gobbi M, Romandini S (1986) Localization of GABAA and GABAB receptor subtypes on serotonergic neurons. Brain Res., 371, 372-375

27. Mos J, Olivier B, Poth M, Van Aken H (1992) The effects of intraventricular administration of eltoprazine, 1-(3- trifluoromethylphenyl)piperazine hydrochloride and 8-hydroxy-2-(di-n- propylamino)tetralin on resident intruder aggression in the rat.

Eur.J.Pharmacol., 212, 295-298

28. Mos J, Olivier B, Poth M, Van Oorschot R, Van Aken H (1993) The effects of dorsal raphe administration of eltoprazine, TFMPP and 8- OH-DPAT on resident intruder aggression in the rat. Eur.J.Pharmacol., 238, 411-415

29. Nishikawa T, Scatton B (1985) Inhibitory influence of GABA on central serotonergic transmission. Raphé nuclei as the neuroanatomical site of the GABAergic inhibition of cerebral serotonergic neurons. Brain Research, 331, 91-103

30. Olivier B, Mos J, Van Oorschot R, Hen R (1995) Serotonin receptors and animal models of aggressive behavior. Pharmacopsychiatry, 28 Suppl 2, 80-90

31. Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates Academic Press, North Ryde, Australia

32. Pellegrino LJ, Cushman AJ (1967) A stereotaxic atlas of the rat brain Appleton-Century-Crofts (Division of Meredith Publishing Company), New York

33. Pineyro G, Blier P (1999) Autoregulation of serotonin neurons: role in antidepressant drug action. Pharmacol.Rev., 51, 533-591

34. Popova NK, Kulikov AV, Nikulina EM, Kozlachkova EY, Maslova GB (1991) Serotonin metabolism and serotonergic receptors in Norway rats selected for low aggressiveness to man. Aggressive Behavior, 17, 207-213

35. Sijbesma H, Schipper J, De Kloet ER, Mos J, Van Aken H, Olivier B (1991) Postsynaptic 5-HT1 receptors and offensive aggression in rats: a combined behavioural and autoradiographic study with eltoprazine. Pharmacol.Biochem.Behav., 38, 447-458

36. Stork O, Welzl H, Cremer H, Schachner M (1997) Increased intermale aggression and neuroendocrine response in mice deficient for the neural cell adhesion molecule (NCAM). Eur.J.Neurosci. , 9, 1117-1125

37. Tao R, Ma Z, Auerbach SB (1996) Differential regulation of 5-hydroxytryptamine release by GABAA and GABAB receptors in midbrain raphe nuclei and forebrain of rats. Br.J.Pharmacol., 119, 1375-1384

38. Tao R, Auerbach SB (2000) Regulation of serotonin release by GABA and excitatory amino acids. J.Psychopharmacol., 14, 100-113

39. Tuinier S, Verhoeven WM, Van Praag HM (1995) Cerebrospinal fluid 5-hydroxyindolacetic acid and aggression: a critical reappraisal of the clinical data. Int.Clin.Psychopharmacol., 10, 147-156

40. Van der Vegt BJ, De Boer SF, Buwalda B, De Ruiter AJ, De Jong JG, Koolhaas JM (2001) Enhanced sensitivity of postsynaptic serotonin-1A receptors in rats and mice with high trait aggression. Physiology & Behavior, 74, 205-211

41. Van Erp AM, Miczek KA (2000) Aggressive behavior, increased accumbal dopamine, and decreased cortical serotonin in rats. J.Neurosci., 20, 9320-9325

42. Van Praag HM (1998) Anxiety and increased aggression as pacemakers of depression. Acta Psychiatr.Scand.Suppl , 393, 81-88

43. Westergaard GC, Suomi SJ, Higley JD, Mehlman PT (1999) CSF 5-HIAA and aggression in female macaque monkeys: species and interindividual differences. Psycho-pharmacology (Berl), 146, 440-446